PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2004/002271	27.05.2004	02.07.2003

International Patent Classification (IPC) or both national classification and IPC
G01N33/68

Applicant
NORDLUND, Pär

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/002271

IAP20 Rec'd PCT/PTO 30 DEC 2005

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	17
Inventive step (IS)	Yes:	Claims	1-16
	No:	Claims	17
Industrial applicability (IA)	Yes:	Claims	1-17
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

   and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

   see form 210

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V.

1 The following documents are referred to in this communication:

D1 : KNAUST R K C ET AL: "Screening for soluble expression of recombinant proteins in a 96-well format" ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS, NEW YORK, NY, US, vol. 297, no. 1, 1 October 2001 (2001-10-01), pages 79-85, XP002240117 ISSN: 0003-2697

D2 : WALDO G S ET AL: "Rapid protein-folding assay using green fluorescent protein." July 1999 (1999-07), NATURE BIOTECHNOLOGY. JUL 1999, VOL. 17, NR. 7, PAGE(S) 691 - 695 , XP002291069 ISSN: 1087-0156

D3: US 2002/127587 A1 (TRINH THUAN ET AL) 12 September 2002 (2002-09-12)

D4: GB-A-2 152 214 (UNIV SURREY) 31 July 1985 (1985-07-31)

2 Novelty, Inventive Step and Industrial Applicability

2.1 D1 discloses a method for identifying well-expressing soluble proteins in E- coli whereby the cells are lysed, the lysate is filtered and the target protein is detected by a reporter function. In contrast to the present application, a 96 well format is used for screening and no whole cell colonies are lysed and examined.
In D2 green fluorescent protein is used as a reporter for soluble proteins, whereby also whole cell colonies are examined (Fig 3A). D2 is different from the present application in that the colonies are not lysed and filtered.
Claims 1-16 are therefore novel over D1-D2.

2.2 Claim 17 relates to an extremely large number of possible products (kits) and lacks novelty for the following reasons:
The feature "support" (item (b)) is not clear in the claim and thus not limiting the scope of the claim. Also the lysis reagent (item (c)) is only optional and thus not limiting the scope of the claim. For the filter membrane described in item (a), it appears that any standard membrane can be used (see p. 9 of the description). Any document that discloses a test kit comprising a filter thus anticipates the novelty of said claim (e.g. D3 or D4), or any product comprising one or more standard filter membranes.

It is noted that also the description fails to give any specific examples of supports that should be present in the kit (only the inclusion of a capture membrane is given

as an example on p. 17, which does itself not have a clear definition). From the rest of the description, it appears that this solid support/capture membrane can be any standard filter membrane.

2.3 Claims 1-16 meet the criteria of Article 33(1) PCT, because the subject matter of said claims involves an inventive step in the sense of Article 33(3)PCT.

Document D1, which is considered to represent the most relevant state of the art to the subject matter of claims 1-16, discloses lysis and filtration of cells of interest. Thereby, cells from plates were picked and cultured in microtiter plates. The identification of the cell colony which expresses the soluble variant of interest is implicit from D1.

The subject-matter of independent claims 1-16 differs from the disclosure of D1 in that:
said cell colony (claims 1-15) or one or more colonies of cells (claim 16) are subjected to conditions which are capable of causing lysis thereof

The technical effect of this feature is that the method thus allows for the direct identification of cell colonies expressing the soluble variant, without need for further culturing the picked colonies in microtiter plates and thus many pipetting steps.

The problem to be solved by the present invention may therefore be regarded as providing a method of simplified screening, which allows for a faster processing of a large number of recombinant clones.

The solution is to directly lyse the colonies and to filter the lysates

None of the prior art documents suggest the present method and its advantages over the prior art document D1. Thus, claims 1-16 meet the requirements of Art. 33(3) PCT.

2.4 Claims 1-17 are considered to be industrially applicable.

Re Item VIII.

1.1 Claim 1 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The following functional statements do not enable the skilled person to determine which technical features are necessary to perform the stated functions: the target is not detected on the basis of its own enzymatic activity. The objection can be overcome by inclusion of the technical features given in the description, which specify how the result is achieved.

1.2 The expression "native lysis" used in claims 2 and 4 is not common in the art and does not have a generally recognised meaning (Art. 6 PCT).
It is noted that from the description it appears that non-denaturing lysis is meant, which is also the term used in D1,

It is furthermore noted that the non-denaturing lysis appears to be an essential feature of the invention, which allows for the separation of soluble proteins. Since independent claims 1 and 16 do not contain this feature, they do not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

1.3 Claim 17 contravenes the requirements of 6 PCT for the following reasons: The feature "support" (item (b)) is not clearly defined. It is noted that the description also fails to give any specific examples of solid supports that should be present in the kit. Only the inclusion of a capture membrane is given as an example on p. 17, which does itself also not have a clear definition. For the above reasons, claim 17 also lacks support in the description as required by Article 6 PCT, as its scope is broader than justified by the description and drawings.

1.4 Although claims 1 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought or appear to be embodiments of the same process. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, said claims do not meet the requirements of Article 6 PCT.

In order to overcome this objection, it would appear appropriate to file an amended set of claims defining the relevant subject-matter in terms of a single independent process claim followed by dependent claims covering features which are merely optional (Rule 6.4 PCT).

1.5 The citation given on p. 3 (Peabody) is wrong.